

CLAIMS

1. Immunoreactive HTLV-III polypeptide expressed by cells transformed with a recombinant vector containing HTLV-III cDNA.
- 5 2. A polypeptide of Claim 1 wherein said HTLV-III cDNA encodes an env gene sequence.
3. A polypeptide of Claim 2 which is immunoreactive with sera of patients with acquired immunodeficiency syndrome or sera
10 containing antibodies to HTLV-III.
4. A polypeptide of Claim 1 wherein said HTLV-III cDNA encodes an env-lor gene sequence.
5. A polypeptide of Claim 4 which is immunoreactive with sera of patients with
15 acquired immunodeficiency syndrome or sera containing antibodies to HTLV-III.
6. A polypeptide of Claim 1 wherein said HTLV-III cDNA is an EcoRI restriction fragment.
7. A polypeptide of Claim 6 which is immunoreactive with sera of patients with
20 acquired immunodeficiency syndrome or sera containing antibodies to HTLV-III.
8. Isolated HTLV-III envelope polypeptide.

9. Isolated HTLV-III polypeptide encoded by an env-lor gene sequence.
10. Isolated cDNA encoding an HTLV-III gene.
11. cDNA of Claim 10 encoding the HTLV-III env gene.
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12. cDNA of Claim 10 encoding the HTLV-III env-lor gene sequence.
13. cDNA of Claim 10 encoding an EcoRI restriction fragment of HTLV-III cDNA which encodes a polypeptide immunoreactive with sera of patients with acquired immunodeficiency syndrome or sera containing antibodies to HTLV-III.
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14. Isolated cDNA encoding for an HTLV-III polypeptide which is immunoreactive.
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15. Isolated cDNA of Claim 14 encoding for an envelope polypeptide which is immunoreactive with sera of patients with acquired immunodeficiency syndrome or sera containing antibodies to HTLV-III.
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16. Isolated cDNA of Claim 14 which is an EcoRI restriction fragment.
17. A DNA probe comprising a DNA sequence which is essentially homologous to a portion of the HTLV-III genome unique to the virus.
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18. A DNA probe of Claim 17 wherein the DNA sequence is essentially homologous to a portion of the HTLV III genome which encodes a polypeptide immunoreactive with sera of AIDS patents.
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19. A hybrid protein comprising an HTLV-III polypeptide linked to at least one other polypeptide.
20. A hybrid protein of Claim 19 comprising an HTLV-III polypeptide linked to an indicator polypeptide.
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21. A hybrid protein of Claim 20 wherein said indicator polypeptide comprises beta-galactosidase.
- 15 22. An isolated RNA transcript of the env gene of HTLV-III.
23. An isolated RNA transcript of Claim 22 having a label which emits a detectable signal.
24. An isolated RNA transcript of Claim 23 wherein said label comprises a radioisotope.
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25. A recombinant vector containing HTLV-III DNA capable of expression upon insertion into host cells.
26. OmpA vector containing HTLV-III cDNA.

27. pMR 100 vector containing HTLV-III cDNA.
28. A method of producing HTLV-III polypeptide, comprising the steps of:
- a. cleaving HTLV-III cDNA to produce cDNA fragments;
 - b. inserting the cDNA fragments into an expression vector to form a recombinant vector;
 - c. transforming an appropriate host cell with the recombinant vector; and
 - d. culturing the transformed host cell under conditions sufficient for expression of the polypeptide encoded for by the inserted HTLV-III cDNA.
29. A method of Claim 28 wherein the cleaving step comprises digesting the HTLV-III cDNA with restriction endonucleases to produce restriction fragments of cDNA.
30. A method of Claim 28 wherein the cleaving step comprises shearing the HTLV-III cDNA to produce cDNA fragments.
31. A method of producing HTLV-III envelope polypeptide, comprising the steps of:
- a. cleaving HTLV-III genomic cDNA with the restriction endonuclease SstI;
 - b. digesting the cleaved cDNA with restriction endonucleases sufficient to generate restriction fragments which encompass at least a portion of the env gene;
 - c. isolating the restriction fragments;

- d. producing DNA fragments of about 200-500 base pairs in length from the restriction fragments;
 - e. isolating the DNA fragments of about 200-500 base pairs;
 - f. inserting the isolated fragments into the open reading frame expression vector pMR100 for production of hybrid proteins comprising an env gene product and beta-galactosidase;
 - g. transforming lac z⁻ E. coli cells with the vector;
 - h. plating the transformed cells on MacConkey agar plates, maintaining the plates under conditions sufficient for the formation of colonies and selecting cell colonies exhibiting a red color;
 - i. culturing transformed cells from the selected colonies under conditions which allow expression of the hybrid protein;
 - j. obtaining cellular protein from the cultured transformed cells;
 - k. separating the cellular protein obtained;
 - l. contacting the separated protein with sera from AIDS patients to identify protein which is immunoreactive with the sera; and
 - m. isolating the immunoreactive protein.
32. A fusion protein produced by the method of Claim 31.
33. A method of Claim 31, further comprising the step of separating the env gene expression

product from the remainder of the hybrid protein.

34. A HTLV-III envelope polypeptide produced by the method of Claim 33.
- 5 35. Antibody specifically reactive with HTLV-III envelope polypeptide.
36. An antibody of Claim 35 which is monoclonal.
37. Antibody specifically reactive with HTLV-III polypeptide produced by recombinant DNA techniques.
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38. An antibody of Claim 37 which is monoclonal.
39. An immunoassay for the detection of HTLV-III employing antibody which reacts specifically with HTLV-III polypeptide produced by recombinant DNA techniques.
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40. An immunoassay for the detection of HTLV-III employing antibody which reacts specifically with HTLV-III envelope polypeptide.
41. An immunoassay of Claim 40 wherein said antibody is monoclonal.
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42. A sandwich type immunoradiometric assay for the detection of HTLV-III employing an immobilized antibody which reacts with HTLV-III polypeptide

and a soluble antibody which reacts with HTLV-III polypeptide.

43. An assay kit comprising an antibody which reacts specifically with HTLV-III polypeptide bound to a solid phase and a labeled soluble antibody which reacts specifically with HTLV-III polypeptide.
44. A method of detecting antibodies against HTLV-III in a bodily fluid comprising the steps of:
- a. contacting an immunoabsorbent comprising an HTLV-III polypeptide bound to a solid phase with a bodily fluid until antibodies against HTLV-III polypeptide in the bodily fluid bind the solid phase polypeptide;
 - b. separating the immunoabsorbent from the bodily fluid;
 - c. contacting the immunoabsorbent with a labeled HTLV-III polypeptide or labeled antibody against human immunoglobulin; and
 - d. determining the amount of labeled polypeptide bound to immunoabsorbent as an indication of antibody to HTLV-III.
45. A kit for determining the presence of antibody against HTLV-III in a bodily fluid comprising:
- a. an immunoabsorbent comprising a HTLV-III polypeptide bound to a solid phase; and
 - b. labeled HTLV-III polypeptide or a labeled antibody against human immunoglobulin.

46. A method of detecting HTLV-III nucleic acid in a bodily fluid comprising the steps of:
- a. adsorbing the nucleic acid in a bodily fluid onto an adsorbent;
 - 5 b. denaturing the adsorbed nucleic acid;
 - c. contacting the adsorbed nucleic acid with a HTLV-III DNA or RNA probe; and
 - d. determining if the probe hybridizes with the adsorbed nucleic acid.
- 10 47. A method of Claim 46 wherein the bodily fluid is a cell lysate.
48. A hybridoma cell line which produces antibody specifically reactive with HTLV-III envelope polypeptide.